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Platelet Function Abnormalities in Gaucher Disease Patients

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Bleeding manifestations are common in Gaucher disease patients. Although usually attributed to thrombocytopenia, some patients with relatively high platelet counts and normal coagulation tests have hemorrhagic phenomena. To investigate whether perturbed platelet function could explain these bleeding manifestations we performed platelet aggregation tests on 32 type I adult Gaucher patients who were not severely thrombocytopenic (platelet counts >50 × 10^9/L). Seven patients (22%) had abnormal platelet aggregation. In five, platelet aggregation was markedly reduced in response to collagen and ADP and virtually absent in response to epinephrine, whereas two patients had isolated severely impaired epinephrine-induced aggregation. In one patient platelet aggregation markedly improved following one year of enzyme replacement therapy. Incubating normal platelets with high concentrations of glucocerebroside did not impair their ability to aggregate, suggesting that plasma glucocerebroside does not directly interfere with platelet function. Platelet dysfunction is a hithero unrecognised, relatively common cause of excessive bleeding in Gaucher patients. Am. J. Hematol. 61:103–106, 1999.

Key words: Gaucher disease; platelet aggregation; platelet dysfunction; enzyme replacement therapy; imiglucerase

INTRODUCTION

Gaucher disease, the most common lysosomal storage disease, is characterised by accumulation of glucocerebroside in the reticuloendothelial system due to an inherited deficiency of the lysosomal enzyme glucocerebrosidase. In the most common form, type I, involvement is largely limited to the spleen, liver, and bone [1]. Hemorrhagic phenomena are common, being the presenting symptom in 43% of patients in one series [2]. Bleeding manifestations are commonly attributed to thrombocytopenia secondary to infiltrative splenomegaly [1,3]. Deficiencies of various coagulation proteins have also been described [3,4]. However, excessive bleeding has been noted in some patients with platelet counts of more than 100 × 10^9/L and normal clotting studies [5], suggesting perturbed platelet function.

To address this issue we studied platelet function in a cohort of type I Gaucher patients. We found that platelet function abnormalities in Gaucher patients are relatively common, in some cases being the sole explanation for excessive bleeding.

MATERIALS AND METHODS

Patient Population

Consecutively available adult Gaucher patients attending the Shaare-Zedek Medical Center Gaucher clinic were studied. Patients were excluded if they had severe thrombocytopenia (platelet counts of less than 50 × 10^9/L), or were taking medication known to interfere with platelet function. The study was approved by the Shaare-Zedek Institutional Review Board. The mutations in these patients were previously determined, and the extent of organ and bone involvement is expressed by a severity score index (SSI) as previously described [2].

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TABLE I. Clinical and Laboratory Characteristics of Gaucher Patients With Abnormal Platelet Aggregation

Patient No.	Age/Sex	SSI	Bleeding tendency	Spx	Imi	Hb (g/dl)	Plt (10^9/L)	PT (INR)	PTT (sec)	Platelet aggregation studies: Epi	Coll	ADP (2.0 μM)	Ristocetin (0.5, 1.0 mg/ml)
1	42/M	10	None	No	No	13.8	135	0.9	31	<5	50	34	Normal
			post 1 year enzyme replacement therapy							76	66	47	Normal
2	19/F	12	Epistaxis, bruising	No	No	11.4	78	1.4	43	<5	36	40	Normal
3	69/M	4	None	No	No	11.5	91	1.1	37	<5	50	40	Normal
4	44/F	3	Massive postoperative bleeding	No	No	12.9	77	1.5	64	<5	60	<5	Normal
5	23/M	5	None	No	No	14.0	191	1.1	33	<5	<5	50	Normal
6	24/F	10	Severe epistaxis, bruising	No	Yes	12.3	86	1.4	30	<5	Normal	Normal	Normal
7	39/F	14	Menometerorhagia, bruising	No	Yes	12.4	112	1.1	36	<5	Normal	Normal	Normal

Abbreviations: M, male; F, female; SSI, severity score index; Spx, splenectomy; Imi, imiglucerase; PT, prothrombin time; PTT, partial thromboplastin time; Epi, epinephrine; Coll, collagen; ADP, adenosine diphosphate; INR, international normalised ratio.

Materials

Glucocerebroside was purchased from Sigma (St. Louis, Mo.), and was freshly dissolved in dimethylsulfoxide (DMSO).

Platelet Function Studies

Platelet aggregation was performed by standard technique, using citrated platelet rich plasma, in particular in accordance with the recommendations of the British Society of Haematology Task Force on this subject [6]. Aggregations were performed on a two-channel Payton aggregometer (Payton Associates, Scarborough, Canada). Collagen (1.0 μg/ml, final concentration), ADP (1.0 and 2.0 μM), epinephrine (2.5 μg/ml), and ristocetin (0.5 and 1.0 mg/ml) were used as agonists. Normal platelet rich plasma, obtained from healthy laboratory personnel, diluted to similar or lower platelet counts were used as a parallel control in every experiment. Aggregation was considered abnormal if maximum aggregation was less than 50% of the control. Results are expressed as percentage aggregation compared with a normal control. In some experiments normal platelets were incubated with glucocerebroside for various periods of time before performing aggregation.

In addition, complete blood counts and standard coagulation tests (prothrombin time [PT] and partial thromboplastin time [PTT]) were performed on all patients as part of their routine follow-up.

Statistical Analysis

Comparisons between groups were performed using the two-tailed Fisher's exact test.

RESULTS

Thirty-two adult Gaucher patients (seven male and 25 female) were studied, mean age 39 (range 17–69) years. Eleven patients had undergone splenectomy. Twenty-two patients were currently treated with imiglucerase. The mean SSI at presentation was10.9 (range 3–27), suggesting that this cohort had relatively severe disease. Mean platelet count was 180×10^9/L (range $74–508 \times 10^9$/L). Only one patient had an abnormal PT, whereas nine had abnormal PTT.

Seven patients (22%) had abnormal aggregation studies. Five patients had markedly reduced platelet aggregation (<50%) in response to collagen and/or ADP and virtually no aggregation in response to epinephrine. The other two patients had isolated severely impaired (<5%) epinephrine-induced aggregation. In all patients, ristocetin-induced aggregation was normal. Four of these patients had an otherwise unexplained bleeding tendency. In one patient aggregation studies repeated one year after initiating enzyme replacement therapy demonstrated marked improvement. None of the patients with abnormal aggregation had undergone splenectomy, as compared with 44% of the patients with normal studies ($p = 0.066$). Two patients (28%) were receiving enzyme replacement therapy (versus 20/25 [80%] of patients with normal aggregation, $p = 0.018$). Both of these patients had isolated abnormal epinephrine-induced aggregation. Characteristics of these seven patients are detailed in Table I. Representative aggregation assays of a Gaucher patient and a normal control are shown in Figure 1.

In separate experiments aggregation studies were performed on normal platelet rich plasma following incubation with glucocerebroside (final concentrations 0.1–50 μg/ml). No impairment in aggregation was noted with incubation times of up to three hours.

DISCUSSION

Bleeding phenomena in Gaucher patients, the most common presenting manifestation [2], are usually attributed to thrombocytopenia. However, 22% of the Gaucher patients we studied had abnormal platelet function. In a detailed search of the literature we could find only two reports concerning platelet function in Gaucher patients. In the first, the authors described two cases of what they

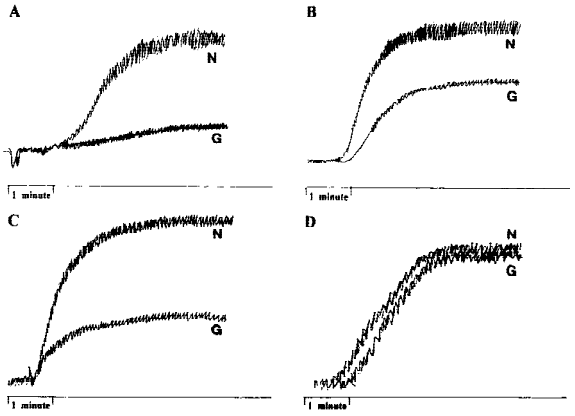

Fig. 1. Platelet aggregation study in a Gaucher patient (G) and a parallel normal control (N) whose platelet rich plasma was diluted to similar platelet counts. Aggregation was performed with epinephrine (2.5 µg/ml, final concentration) (panel A), collagen (1.0 µg/ml) (B), ADP (2 µM) (C), and ristocetin (1.0 mg/ml) (D).

termed "pseudo-pseudo Bernard-Soulier syndrome," i.e., patient plasma inhibited ristocetin-induced aggregation of normal platelets [7]. None of our patients had a similar phenomenon. In another report, platelet aggregation studies were performed in 11 Gaucher patients. Although exact details are not reported, it is stated that two patients had a "storage-pool'-like defect [8].

Could our findings be a laboratory artifact related to the relatively low platelet counts? Ho et al. reported that epinephrine-induced aggregation was reduced to an average of 25% in patients with platelet counts of less than 100×10^9/L [9]. We could not reproduce their findings. Aggregation was completely normal in eight patients with platelet counts of less than 100×10^9/L and in all control platelets diluted to similar counts. Furthermore, four of the seven patients with abnormal aggregation had bleeding manifestations.

It is also unlikely that increased levels of plasma glucocerebroside are directly responsible for the impaired platelet aggregation. It was not possible to induce defective aggregation in normal platelets by incubating them with extremely high concentrations of glucocerebroside (up to 50 µg/ml) for up to three hours. This is more than 1,000 times the concentration in plasma of Gaucher patients [1], and similar to that required to demonstrate inhibition of superoxide generation in normal monocytes [10]. Our experiments do not rule out the unlikely possibility that more prolonged exposure of platelets to high concentrations of glucocerebroside is required for impaired aggregation.

An ongoing low-grade activation of the coagulation system was recently described in Gaucher patients [4]. It is possible that thrombin generation leads to chronic platelet activation resulting in platelet "exhaustion" and hence impaired platelet aggregation. Another possibility is that a macrophage-secretory product is responsible for the impaired aggregation. It is interesting to note that all of the patients with impaired platelet aggregation had an intact spleen and only two were receiving enzyme replacement therapy. In these two patients aggregation was normal with all agonists except epinephrine, suggesting a milder defect. Platelet aggregation studies markedly improved in a single patient following one year of replacement therapy. Taken together these results suggest a connection between total body burden of storage cells and abnormal aggregation studies. Similar findings have been reported with other abnormalities. Clotting factor deficiencies improved following splenectomy [11] or after initiating enzyme supplementation therapy [4]. Impaired neutrophil chemotaxis is corrected with one year of enzyme replacement therapy [12] or following splenectomy.

What are the clinical implications of these abnormalities? Gaucher patients frequently require surgery, including major orthopaedic procedures for disease-related complications. Platelet function defects may potentially contribute to postoperative hemorrhage (such as in patient 4). We do not currently advocate widespread screening of all Gaucher patients for platelet function defects. However, we recommend that platelet function tests be performed on all nonsplenectomised Gaucher patients not receiving enzyme replacement therapy prior to surgical procedures, or with an unexplained bleeding disorder.

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